DEVELOPMENT OF CAMPANIFORM SENSILLA ON THE
WING OF THE TOBACCO HORNWORM, MANDUCA SEXTA

by

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INTRODUCTION

The morphological and physiological development of cuticular sensory receptors in insects has received little attention. Most early studies were purely morphological, utilizing methylene blue and conventional histological techniques, and dealt primarily with the ingrowth of sensory axons to the central nervous system (Wigglesworth, 1953; Clever 1959, 1960; Fudalewicz-Neimczyk and Rosciszewska, 1972b). There have appeared recently several electron microscopic descriptions of receptor development during the molting cycle of some hemimetabolous insects (Gnatzy and Schmidt, 1972 a, b; Altner and Thies, 1972; Schmidt, 1974; Larink, 1976; Moran et al., 1976; Gnatzy, 1978) and two studies on the development of antennal receptors in holometabolous insects (Ernst, 1972; Sanes and Hildebrand, 1976). The physiological response of developing receptors has been investigated primarily during the molting cycles of larvae and of hemimetabolous insects (Haffer, 1921; Richard, 1952; Wigglesworth, 1953; Moran et al., 1976, Gnatzy and Tautz, 1977). The only physiological study concerning development of receptors in a holometabolous insect is that of Schweitzer et al. (1976) on the antennal sensilla of Manduca sexta. They reported that measurable electrophysiological responses to olfactory and mechanical stimuli first occurred during the final week of the pupal period, increasing in magnitude and reaching adult levels one or two days before eclosion. Responses to olfactory and mechanical stimuli did not develop simultaneously, suggesting that different receptor types develop asynchronously.

The campaniform sensillum, a type of cuticular mechanoreceptor common throughout the Class Insecta, occurs at locations subjected to stress and strain (reviewed by McIver, 1975; Laverack, 1976). In the cockroach,

campaniform sensilla provide sensory feedback utilized in control of locomotion (Pearson et al., 1973). Groups of campaniform sensilla near the wing base of locusts help regulate wing twisting and maintain stability during flight (Gettrup, 1966). The distribution of campaniform sensilla on the wing of Manduca sexta has been mapped by Kammer and Athey (personal communications) and is similar to that reported on wings of other Lepidoptera (Vogel, 1911; Clever,1959). The fine structure of campaniform sensilla has been described by other investigators (Stuart and Satir, 1968; Chevalier, 1969; Smith, 1969; Moran et al., 1961, Mueller et al., 1978). A sensillum is composed of a bi- polar sensory neuron and two supporting cells: the trichogen cell (inner enveloping cell), which secretes the cuticle of the dome, and the tormogen cell (outer enveloping cell), which secretes the cuticular rim surrounding the dome (Fig. 1). The sensilla of at least one species has a third supporting cell, the dendritic sheath cell, which surrounds the dendrite (Gnatzy and Schmidt, 1972). The dendrite of the bipolar neuron is attached to the inner surface of a cuticular dome that is surrounded by a raised rim of cuticle. Distally, the dendrite gives rise to a modified cilium, the sensory process, which is surrounded by an extracellular fluid compartment, the receptor lymph space (Fig. 1). A tubular body, characterized by a dense array of microtubules surrounded by a dense amorphous substance (Thurm, 1964), is located at the distal tip of the sensory process. It is believed to play a key role in the sensory transduction process (Moran et al., 1976).

The development of campaniform sensilla was followed by Wigglesworth (1953) in a light-microscopic account of abdominal receptors. He observed that all cells of a single sensillum arise by differentiative divisions of a single generalized epidermal cell. More recently, Moran et al. (1976) examined the fine structural changes and physiological responses of cockroach campaniform sensilla during the molting cycle. They found that campaniform

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Figure 1. Diagrammatic representation of a functional campaniform sensillum.
          This sketch is based on my own observations of adult wing
          sensilla and on information in the literature.

Receptor Lymph Space

Dome

Sensory Process

Rim

Dendrite

Wing Cuticle

Tormogen Cell

Trichogen Cell

Neuron Soma

Axon

10 μm
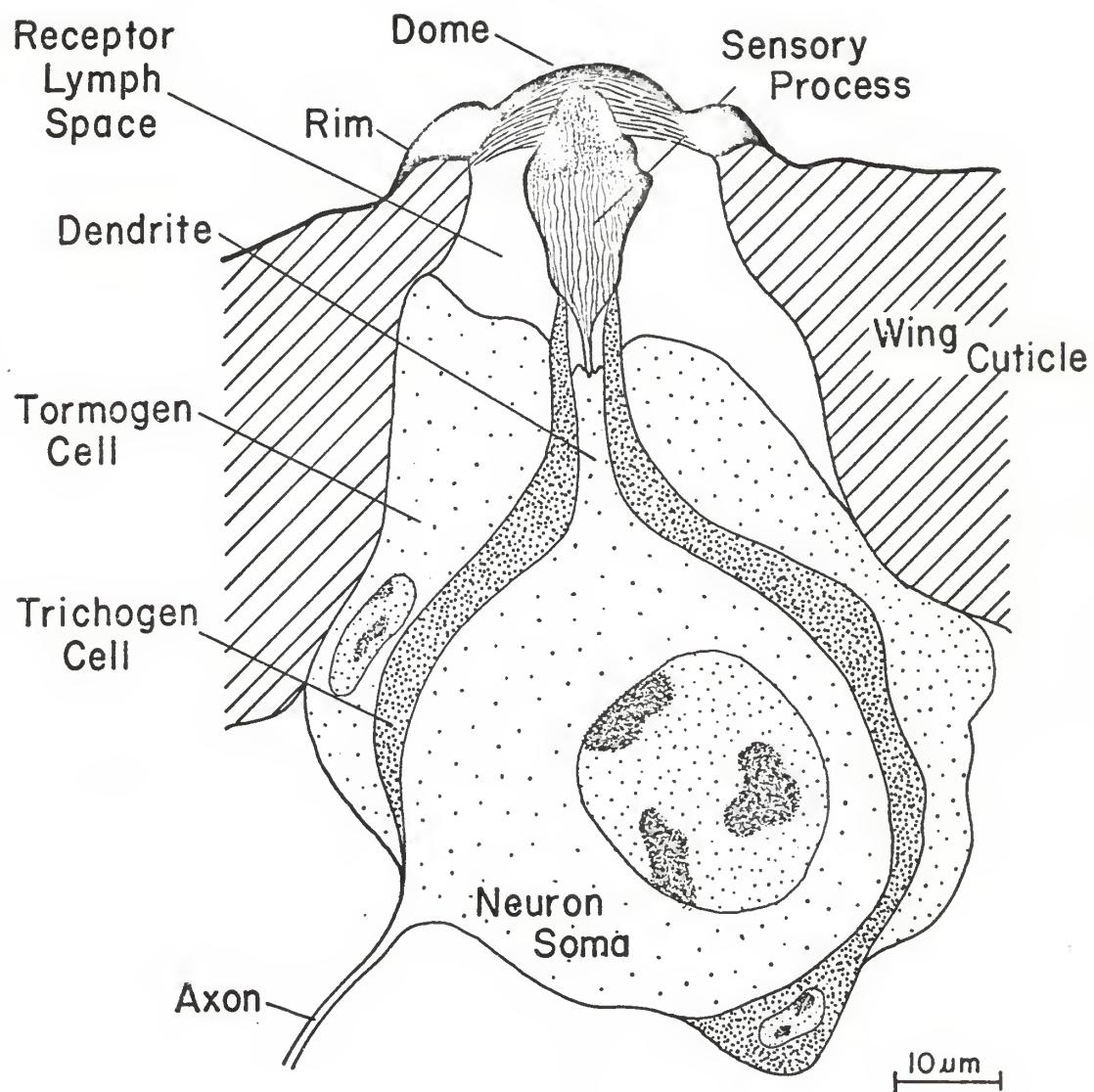
sensilla were physiologically active at all stages of the molting cycle and observed that old and new cuticular domes were functionally connected until molting by an elongation of the sensory process.

The purpose of this study was to investigate development of campaniform sensilla on the wing of the tobacco hornworm moth, Manduca sexta. Except for observations made by Clever (1959, 1960) on methylene blue preparations of Galleria wings, no studies have been performed on the development of mechanoreceptors on the wings of holometabolous insects. The wing of Manduca is an appropriate experimental object for a developmental study because it provides a known array of accessible, widely spaced receptors located on a structure that experiences a relatively long period of development. By examining both responsive and nonresponsive sensilla, it should be possible to observe what morphological features are essential for sensory transduction. This paper presents the results of electrophysiological recording from the developing wing of Manduca sexta and also reports scanning and transmission electron microscopic observations of the developing wing.

MATERIALS AND METHODS

Larvae of Manduca sexta were fed the diet of Baumhover et al. (1977).
After pupation, animals were maintained on a 16 hr light/8 hr dark cycle
with a simultaneous 26°C day/23°C night temperature regimen. On this
schedule the pupal stage lasted 18-20 days; eclosion usually occurred on
the evening of the 19th day. The age of pharate moths was assessed on the
basis of external morphological characteristics (Kammer and Kinnamon, 1979).
Each staged day did not necessarily correspond with a chronological day.
Newly emerged adults could be detected by the butterfly position of the
wings (Truman and Endo, 1974).

Pharate moths staged Day 16 through Day 19 and newly emerged adults
were used for this study. Animals were prepared for electrophysiological
recording by carefully dissecting away the pupal cuticle overlying the
developing adult wing, spreading the forewing with ventral surface upper-
most on a supporting block of wax, and lightly coating the wing with
petroleum jelly to prevent dessication and consequent wrinkling. Recordings
from both coated and uncoated wings showed no difference in receptor activity,
but coated preparations survived several hours longer than did uncoated
preparations. To record from newly emerged adults, the head and abdomen
were ligated with thread to reduce eclosion movements and prevent wing
inflation.

The electrical activity of sensory axons was recorded with electrodes
made from fine, stainless steel insect pins (size 00). The electrodes,
mounted on a Narishige manipulator and inserted into a wing vein, detected
activity in the nerve formed by the confluence of axons of all sensilla,
both hair and campaniform, along that vein. The anatomy of the wing
determined the proximal limit of accessibility to recording electrodes

(Fig. 2). Receptors were stimulated by deforming the forewing cuticle using a hand-held probe formed from a piece of capillary tubing drawn to a tip of approximately 0.25 mm. Since I was not interested in changes in receptor sensitivity, no attempt was made to define more precisely the parameters of stimulus application. Signals from the receptors were amplified by a Grass P15 differential preamplifier, displayed on a Tektronix 5103N storage oscilloscope, and monitored by loudspeaker.

Transmission electron microscopic observations were made on the pair of campaniform sensilla at the distal end of veins R_5 through Cu_{1b} (Fig. 2). Those sensilla were chosen because they could be excised easily and because thinness of the distal cuticle facilitated fixation and sectioning. Pharate moths of staged Day 16 and Day 19 were examined. Both non-responsive receptors from early Day 19 animals and responsive receptors from late Day 19 animals were examined. Late Day 19 animals were electrophysiologically tested for responsive sensilla to insure that functional receptors were fixed. Pieces of wing (Fig. 2) were fixed on ice 24-60 hr in 3% glutaraldehyde in Sorensen's phosphate buffer (pH 7.3) with 2.5% sucrose. Observations were also made on sensilla fixed in 5% glutaraldehyde-2% acrolein and in Karnovsky's fixative (Karnovsky, 1965) to insure that results were not an artifact of improper fixation. All three fixatives provided similar results. Tissues were washed overnight in cold buffer plus sucrose and postfixed on ice 1.5 hr in buffered 1% OsO_4 with 5% sucrose, then rinsed in distilled water and dehydrated in a graded ethanol series. Following extended infiltration (3-4 days) in Spurr's resin (Spurr, 1969) to prevent separation of cuticle from resin during sectioning, tissue blocks were embedded in Spurr's and cured overnight at 65-70°C. Silver to gray sections.

Figure 2. Schematic drawing of the forewing of <u>Manduca</u> <u>sexta</u> indicating the location of campaniform sensilla. Drawing is based on scanning electron micrographs of the adult wing. Recording electrodes were inserted in veins distal to the dashed line; the distal segments excised for transmission electron microscopy are indicated by dotted lines. Veins: C, costa; Sc, subcosta; R, R_4, radius; M_1, M_2, M_3, median; Cu, Cu_{1a}, Cu_{1b}, cubitus; A, anal; ● , dorsal receptor; ◉ , dorsal receptor group; ○ , ventral receptor; ◯ , ventral receptor group.
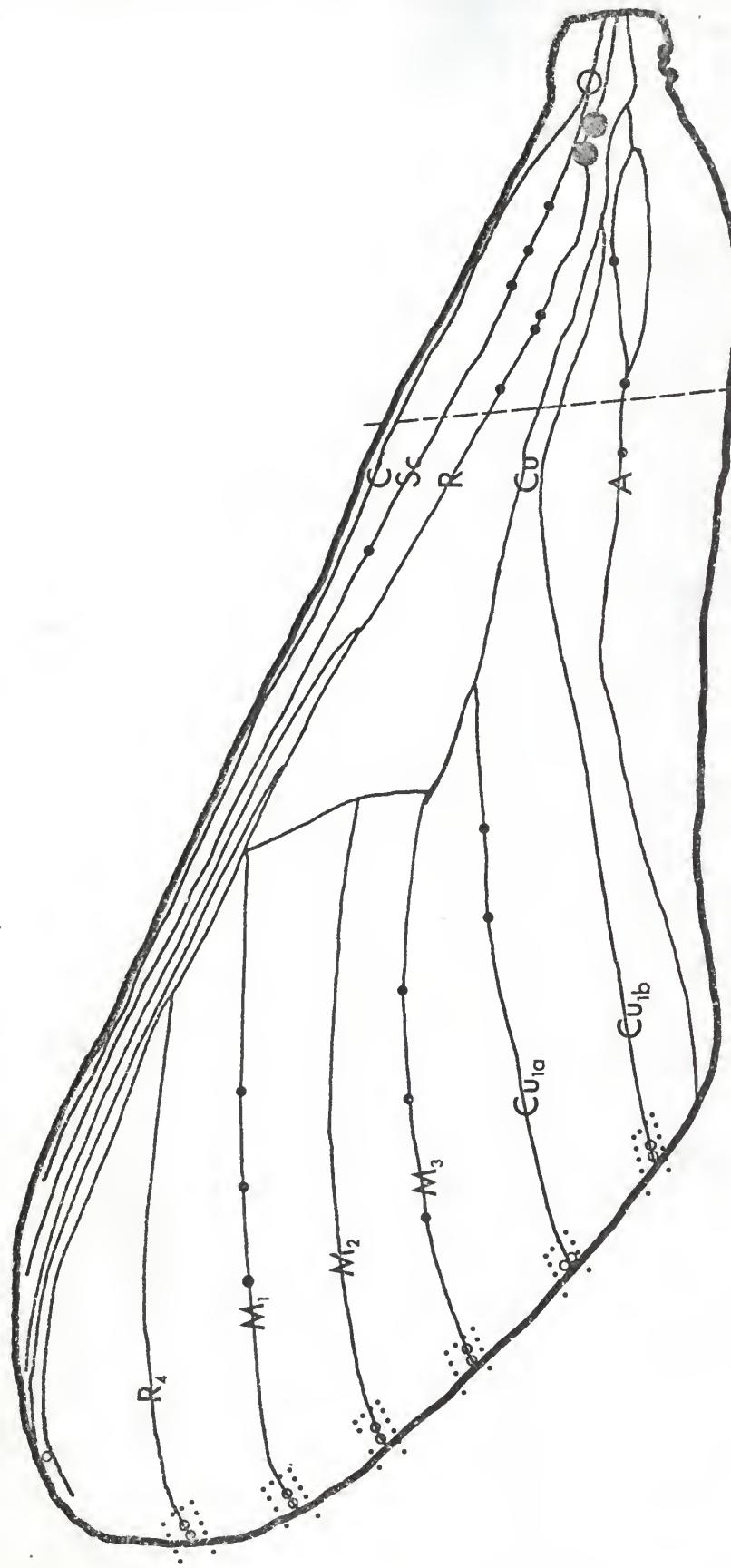
were cut on a Reichert OM U2 Ultramicrotome with a diamond knife and stained 1 hr in 5% ethanolic uranyl acetate and 15 min in lead citrate (Reynolds, 1963). Grids were examined using a Phillips 201 operated at 60 kV. Scanning electron microscopic observations were made on portions of wing fixed as described for transmission electron microscopy. The tissues were critical-point-dried, coated with gold-palladium, and observed using an ETEC Autoscan electron microscope.

RESULTS

Electrophysiology

Campaniform sensilla on the adult wing of Manduca sexta are primarily phasic and characteristically produce a single burst of impulses in response to application of a stimulus. A typical recording from an adult campaniform sensillum is shown in Figure 3 for comparison with receptors on the developing wing.

Recordings made from the subcostal through cubital veins showed that the percentage of responsive sensilla increased from Day 15 until emergence of the adult moth (Table 1). Before Day 15, no sensory activity could be detected by extracellular recording from nerves in veins in the distal two-thirds of the wing.

The first appearance of recordable sensory activity from the distal two-thirds of the wing was noticed in late Day 15 pharate adults. Activity was restricted to the middle third of the wing and was of very small amplitude (10–50 μV). Activity could not be localized to any of the mapped campaniform sensilla and was thus attributed to sensory hairs that are common along the veins of insect wings (Vogel, 1911; Fudalewicz-Niemczyk and Rosciszewska, 1972a; Albert, 1976), and have been observed in scanning electron micrographs of the Manduca wing.

Campaniform sensilla located near the middle of the wing and along veins M_1, M_3, and Cu_{1a} responded to stimulation by late Day 17, but the distal pairs of sensilla were still unresponsive on all veins (Table 1). During Day 19, increasing numbers of animals demonstrated physiologically functional sensilla, and all posteclosion adults with expanded wings exhibited responsive midwing sensilla. Receptors on different veins, but at approximately the same position along the wing's proximal-distal axis, became active concurrently.

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Figure 3. Response of sensilla to stimulation. Scale, 100 mS, A-C;

200 mS, D.

A. Adult--Distal
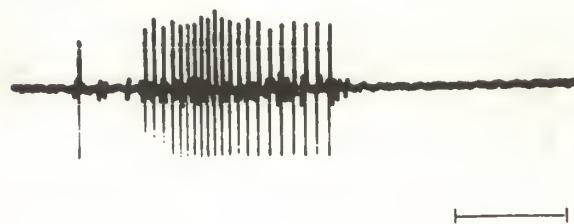
B. Day 16--Proximal

C. Day 18--Midwing

D. Day 19--Distal

Table 1. Times of onset of response to stimulation for midwing and
          distal campaniform sensilla. A probit analysis and t-test
          on the mean time of responses indicates that the difference
          between midwing and distal sensilla is significant at the
          $p < .001$ level.

TABLE 1

| Stage	MIDWING CAMPANIFORM SENSILLA		DISTAL CAMPANIFORM SENSILLA	
	No. of animals	Percent of veins sampled (N) having (a) responsive sensilla	No. of animals	Percent of veins sampled (N) having responsive sensilla
Day 15	4	0 (13)	3	0 (22)
Day 16	4	0 (17)	3	0 (26)
Day 17	5	14 (7)	6	0 (26)
Early Day 18	3	40 (10)	3	0 (21)
Late Day 18	6	52 (21)	8	11 (27)
Early Day 19	5	60 (10)	11	19 (59)
Late Day 19	4	85 (13)	5	55 (22)
Emerged < 1½ Hr	7	94 (17)	12	90 (40)

(a) only veins M_1, M_3 and Cu_{1a} were sampled.

During recording from sensory nerves in the wing veins, several types of apparently spontaneous activity were occasionally noticed. Spontaneous activity was extremely variable in appearance, sometimes occurring as single, irregular spikes or as irregularly spaced bursts. Most noticeable, however, was the occurrence of very regularly spaced bursts of activity characterized by a decreasing spike frequency at the end of each burst. In one animal, spontaneous rhythmic bursting continued 4 hours, but more commonly the bursting activity disappeared within 2 to 5 minutes. Stimulation typically had no effect on the rhythm or duration of bursting, but occasionally halted rhythmic activity. Spontaneous, rhythmic bursting was not seen in adult receptors.

The distal pairs of campaniform sensilla were the last to respond to stimulation. Activity could not be elicited from these receptors before Day 18, with the first pairs becoming functional in late Day 18 pharate moths. More of the distal sensilla became functional during Day 19, but there were not as many sensilla responding distally as proximally (Fig. 4). Throughout the recording process, care was taken to maintain the same sensillum-to-electrode distance, whether recording from distal or midwing sensilla. Thus, the earlier responses of proximal sensilla cannot be attributed to their axons reaching the electrodes earlier.

Campaniform sensilla on the developing wing appeared to respond in a manner similar to that of receptors on the adult wing. However, to substantiate this observation, further experiments are necessary to quantify the response parameters involved. I am unable to explain the apparent increase in amplitude of extracellularly recorded bursts shown in Figure 3.

Figure 4. Graphic representation of onset of receptor response. Data

from Table 1. E, early, L, late; Em, emerged adult.
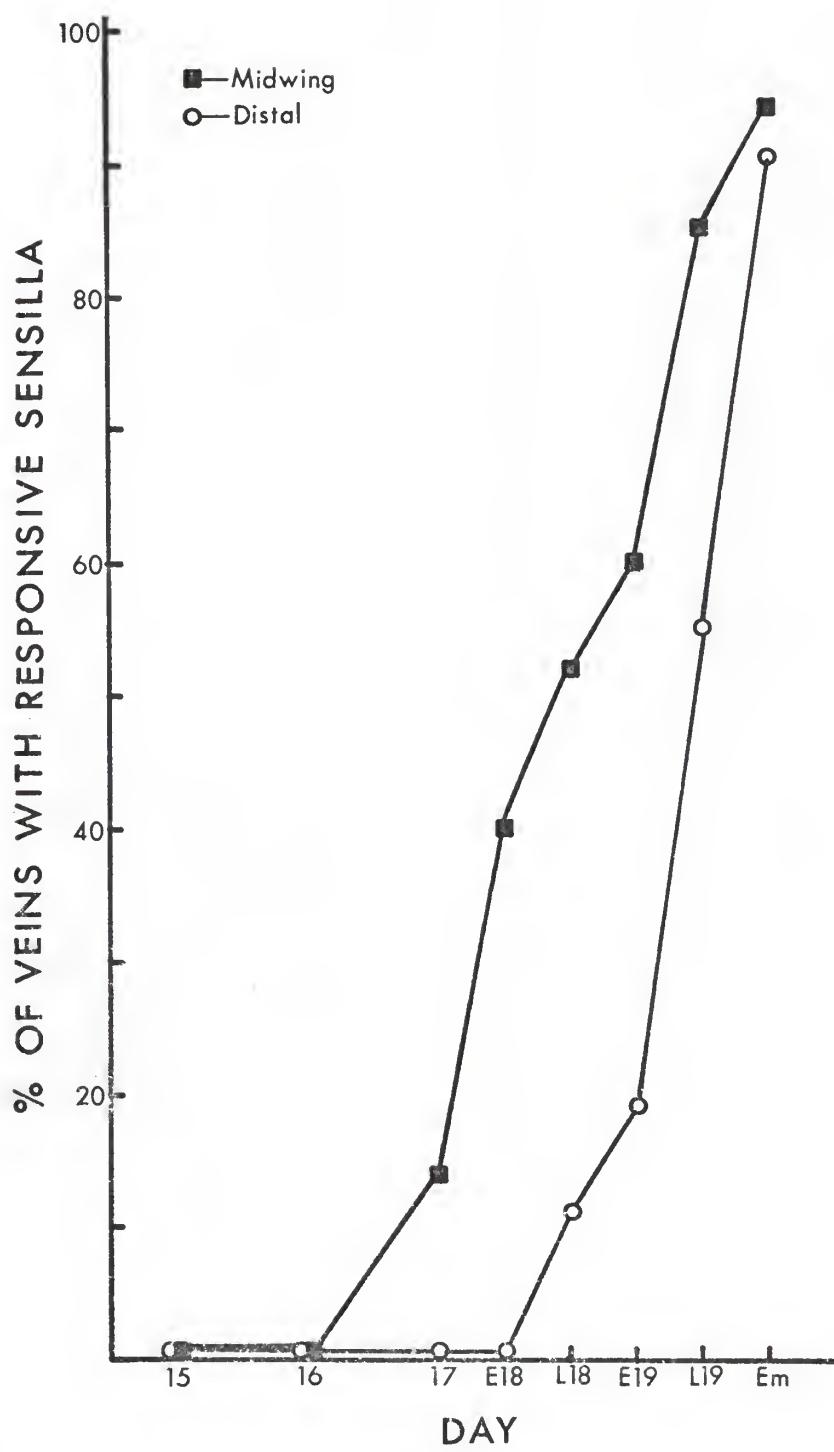
My observations on the wing nerves and Sanes and Hildebrand's (1976)
observations on the antennal nerve of Manduca indicate no increase in
axon diameters between Day 16 and eclosion, so the increased amplitude
is not due to axonal growth.

Microscopy

Scanning electron micrographs of the distal pairs of campaniform
sensilla of Day 16 pharate adults showed an externally morphologically
complete cuticular apparatus (Fig. 5). The convex hemisphere of the dome
with its surrounding cuticular rim was approximately 20 μm in diameter,
similar to that of adult receptors at the same location (Kammer and Athey,
personal communication). The lack of any change in external dimensions is
particularly interesting inasmuch as the wing as a whole undergoes a twofold
increase in length during expansion immediately after eclosion.

Transmission electron micrographs of Day 16 distal nonfunctional
receptors indicated the distal tip of the sensory process was morphologically
complete (Fig. 6). The tip appeared to be securely attached to the inner
surface of the overlying cuticular dome by an electron dense material.
The tubular body with its array of microtubules and associated intracellular
electron dense substance was present. The accessory cells extended to the
cuticle of the dome; the receptor lymph space was conspicuously absent.
Micrographs of nonfunctional receptors from early Day 19 pharate moths
showed no morphological differences from Day 16 animals.

Transmission electron micrographs of functional campaniform sensilla
from late Day 19 pharate adults demonstrated a receptor lymph space surrounding
the distal portion of the sensory process (Fig 7). The appearance of the

Figure 5. Scanning electron micrographs of a distal pair of campaniform
sensilla. A, Day 16 pharate adult. B, mature adult with
expanded, tanned wings. Scale, 10 µm. There is considerable
variation in intersensillum distance among individuals, so
the difference between A and B should not be attributed to the
developmental stage of the animals.
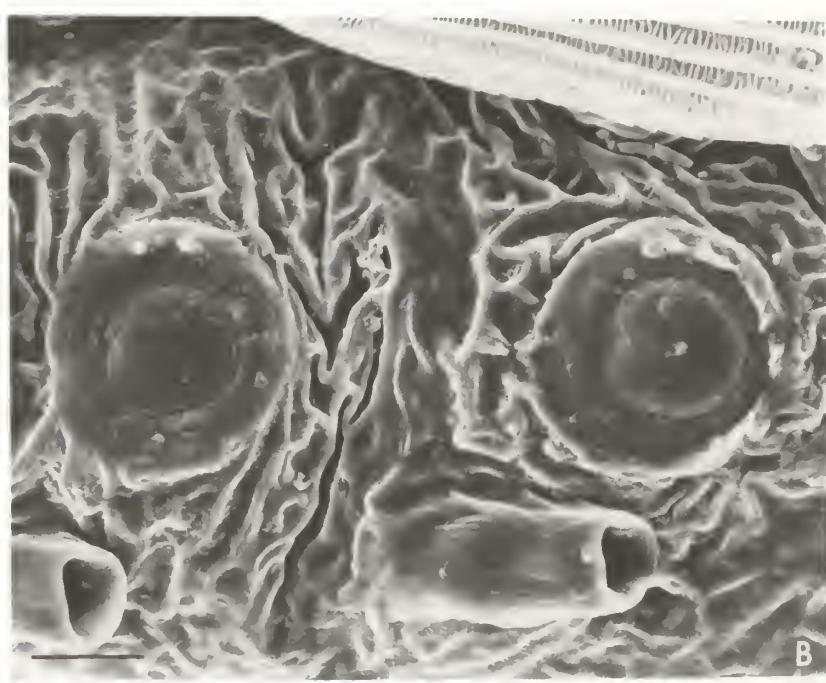
Figure 6. Transmission electron micrograph of a nonfunctional distal
          campaniform sensillum from a Day 16 pharate adult. Oblique
          section through the distal portion of the sensory process.
          Note the absence of the receptor lymph space; instead, the
          cytoplasm of the accessory cells extends to the cuticle of
          the overlying dome. L_1, L_2, layers of cuticular dome; Ac,
          accessory cell; Tu, tubular body at distal tip of sensory
          process. Scale, 1 µm.

Figure 7. Transmission electron micrograph of a functional distal
          campaniform sensillum from a late Day 19 animal. The
          cytoplasm of accessory cells has retracted, resulting in
          formation of a receptor lymph space. L_2, inner layer of
          cuticle; RL, receptor lymph space; Lm, lamellae of accessory
          cells; SP, sensory process; R, ciliary rootlet; D, dendrite.
          Scale, 1 µm.
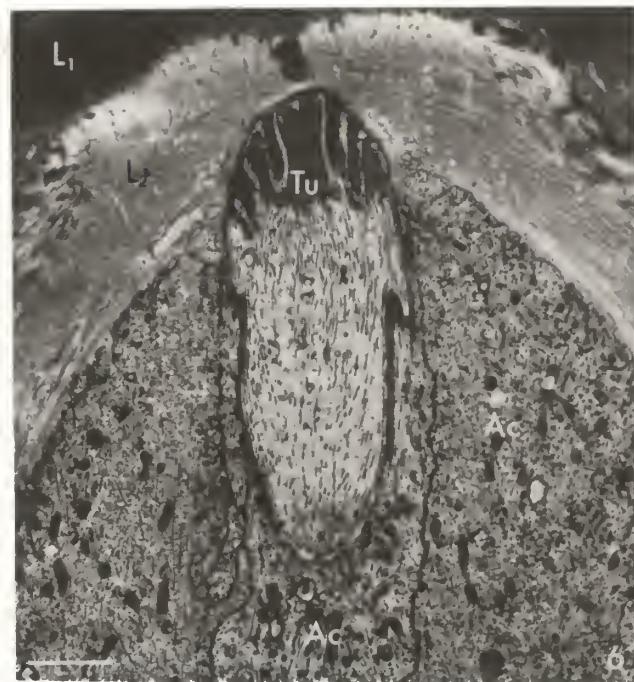
receptor lymph space was apparently due to withdrawal of the accessory cells during the 12-24 hr immediately preceding emergence of the adult moth. Similar observations were made on receptors fixed by several different methods and fixatives, suggesting that the observed withdrawal is not an artifact due to improper fixation.

DISCUSSION

Campaniform sensilla on the developing adult wing of <u>Manduca</u> <u>sexta</u>
become active in a proximal to distal sequence. Receptor groups at the
base of the wing respond to stimulation, or are spontaneously active, as
early as Day 10 or 11 (Kammer and Athey, personal communications). Results
of this study indicate that campaniform sensilla near the middle of the wing's
proximal-distal axis become physiologically functional in late Day 18
pharate adults, whereas receptors along the distal margin do not become
active until late Day 19. The only morphological difference between non-
functional and functional receptors is the presence of a receptor lymph
space in functional receptors.

Results indicate a wide variability in the extent of development of
receptors on the wing of <u>Manduca</u>. Distal campaniform sensilla are responsive
as early as Day 18 in some animals but do not respond in most animals until
after emergence and wing expansion, 24-36 hr later. Variability can
probably be attributed to individual variation in development. Similar
variability has been observed in amphibian Merkel cell development (Tweedle,
1978) and Pacinian corpuscle development in the cat (Malinovsky, 1976).

Onset of Response to Stimulation

One question raised by results of this study is what factor(s) determine(s)
when an individual campaniform sensillum becomes responsive to stimulation,
as measured by its ability to produce measurable action potentials.

The observation that campaniform sensilla are responsive before
emergence of the adult moth indicates that tanning of the wing cuticle is
not required for receptor activity. That is surprising because it is
presumed that mechanical properties of the dome and rime cuticle are involved

in directionality and sensitivity of stimulus reception (Chapman and Duckrow, 1975). Compliance and other mechanical properties of the cuticle would be expected to change during the tanning process, and these changes should influence the response characteristics of a campaniform sensillum. Although a detailed analysis of response characteristics was not performed, no obvious changes were noted between those of pharate adults and those of emerged adults. The lack of change in dimensions of the cuticular apparatus during wing expansion (Fig. 5) suggests that the dome and rim are less extensible than the surrounding wing and perhaps already tanned. Further experiments measuring compliance and sensitivity of the cuticular apparatus of pharate and mature adult sensilla are necessary to substantiate this suggestion.

The lack of a recordable response could be attributed to axonal immaturity. However, observations of nerves in the wing veins (data not shown) indicate that axons appear morphologically mature by Day 16. Schweitzer et al. (1976) made similar observations on antennal sensilla development in Manduca.

If a campaniform sensillum is physiologically functional, the sensory transduction apparatus that transforms the mechanical energy of cuticular strain into electrical potentials must also be functional. The exact site and mechanism of sensory transduction in a campaniform sensillum are unknown. Moran and Varela (1971) demonstrated that microtubules in the sensory process are essential for receptor function and have localized the site of transduction to the distal tip of the sensory process which contains the tubular body (Moran et al., 1976). Ultrastructural changes in the tubular body of blowfly mechanoreceptors have been noticed when these receptors are fixed in a stimulated state (Matsumoto and Farley, 1978).

Thurm (1964) has suggested that compression of the tubular body is a key step in the transduction process in honey bee hair-plate receptors, which are homologous to campaniform sensilla (Lees, 1942; Schwartzkopff, 1974). In developing campaniform sensilla on the wing of Manduca, both the site of stimulus reception (cuticular cap and rim) and the presumed site of sensory transduction (tubular body) appear to be completely formed and securely connected several days before the sensilla respond to stimulation (Fig. 6). These observations suggest that the presence of these structures is not a sufficient condition for receptor responsiveness.

The only morphological difference observed in this study between non-functional and functional receptors is a receptor lymph space in functional sensilla. Retraction of the trichogen and tormogen cells to form the receptor lymph space has been observed in cockroach campaniform sensilla (Moran et al., 1976) and cricket filiform hairs (Gnatzy, 1978) during the molting cycle, and was presumed to occur in dipteran haltere campaniform sensilla (Chevalier, 1969). Inasmuch as the contents of the receptor lymph space bathe the dendritic process, the ionic composition or concentration of this fluid would be expected to be involved in the transduction process. Thurm (1972, 1974) discussed the possible role of the receptor lymph in generation of the transepithelial potential measured across the cuticle at sites of campaniform sensilla in Musca. His data suggest that the apical membrane of the tormogen cell actively transports ions into the receptor lymph space. Gnatzy and Weber (1978) provide similar evidence for olfactory sensilla in Calliphora. In Manduca, establishment of the proper ionic milieu for the receptor potential could follow or accompany retraction of non-neural supporting cells. This could explain why campaniform sensilla do not respond to stimulation until after appearance of the receptor lymph space.

Proximal-Distal Sequence of Sensory Competence

A second question raised by results presented here is what factors are responsible for the proximal-distal sequence in which campaniform sensilla become capable of responding to cuticular stress?

One obvious difference between proximal and distal receptors is the distance that sensory axons must grow before contacting their normal synaptic targets in the central nervous system. Assuming that axon out- growth begins at the same time, as is the case in developing antennal receptors, and proceeds at the same rate for both proximal and distal neurons (Sanes and Hildebrand, 1976), proximal axons would be expected to reach the central nervous system before distal axons. Formation of central synaptic connections could then initiate a sequence of events culminating in physiological maturity of the sensory neurons. Dependence of neurons on normal synaptic formation for proper development has been well documented in both vertebrate and invertebrate nervous systems (reviewed by Hughes, 1968; and Jacobson, 1978). Several types of vertebrate mechanoreceptors fail to differentiate normally when deprived of sensory innervation during develop- ment (Zelena, 1976). However, Edwards et al. (1978) have shown that all cuticular sensory structures, including campaniform sensilla, exhibit normal morphological development in cultured Drosophila leg and wing imaginal discs which lack central synaptic targets. Furthermore, Sanes et al. (1976) reported that removal of the pupal brain in Manduca, before antennal sensory axons could form normal synaptic contacts, had no effect on subsequent morphological, biochemical, or physiological development of antennal sensory neurons. Clever (1960) cut and blocked selected veins on developing Galleria wings, thus preventing sensory axons from making normal synaptic contacts, and observed normal morphological development of sensory bristles and campaniform sensilla.

On the wing of Manduca, the midwing and distal campaniform sensilla that were tested for activity are separated by about 1 cm. The actual rate of axonal elongation and time of arrival at the central nervous system are not known, but at a maximum rate of 1-2 mm per day (Jacobson, 1978), a difference of 5 days between the arrivals of proximal and distal axons at the central nervous system would be expected. However, a separation of only 12-24 hr was observed between proximal and distal sensilla in attainment of sensory competence. Thus it appears that a difference in time of arrival at the central nervous system is not a reasonable explanation for the observed difference in onset of receptor responsiveness.

The observed proximal-distal sequence of sensilla maturation could be explained on the basis of a proximal-distal developmental gradient along the wing. Gradients have previously been proposed to explain specification of polarity and positional information in the integument of insects (reviewed by Bate and Lawrence, 1973). The ripple pattern of the adult cuticle of Rhodnius has been extensively investigated in this respect. The orientation of ripples indicates the polarity of underlying epidermal cells that secrete cuticle. Locke (1959), in a series of graft transplantation and rotation experiments, found that establishment of the pattern was consistent with existence of an anterior-posterior gradient of positional information that was repeated within each abdominal segment. Lawrence et al. (1972) extended these studies using computer modeling and perceived that the results could be explained on the basis of an assumed concentration gradient of a diffusible morphogen. During very early development in Drosophila, acquisition of competence within the immature eye-antennal imaginal disc follows a proximal-distal pattern that is similar to the temporal sequence of differentiation events that occur during adult development (Gateff and Schneiderman, 1975).

Observations by Nardi and Kafatos (1976a, b) of scale cell polarity in transplanted grafts in the wing epidermis of Manduca provide convincing evidence for existence of a proximal-distal morphogenetic gradient along the wing. Greenstein (1971a) noticed a proximal-distal temporal difference in deposition of cuticulin by generalized epithelial cells along the wing of the giant silkmoth, Hyalophora cecropia. By Day 5 of Hyalophora's 21-day pupal period, he observed that epidermal cells in proximal portions of the wing were at a more advanced stage of cuticulin deposition than were cells in distal regions. He did not mention any regional differences in the development of scale- and socket-forming cells (Greenstein, 1971b).

The bipolar neuron of the campaniform sensillum, along with the trichogen and tormogen cells that secrete the specialized cuticular structures of the sensillum, originate by differentiative divisions from generalized epidermal cells (Wigglesworth, 1953). If epidermal cells at the base of the wing develop earlier than those distally, as suggested by Greenstein (1971a), the differentiative divisions giving rise to campaniform sensilla, and subsequent maturation of sensilla, should also occur earlier proximally. This would explain why proximal sensilla become physiologically active before distal sensilla. However, the causal mechanism remains unknown.

In summary, observations from electrophysiologically recorded sensory activity along the wing of Manduca sexta indicate that campaniform sensilla near the middle of the wing's proximal-distal axis become responsive to stimulation the day before eclosion, whereas the sensilla along the wing's distal margin do not respond until late on the day of eclosion. Scanning and transmission electron microscopy indicate that the site of stimulus reception and presumed site of sensory transduction are morphologically

complete several days before a campaniform sensillum attains physiological maturity. The only morphological difference between responsive and unresponsive receptors is the appearance of the receptor lymph space in functional sensilla, which suggests that this feature plays a key role in the transduction process. Results also support the existence of a proximaldistal developmental gradient along the wing.

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DEVELOPMENT OF CAMPANIFORM SENSILLA ON THE
WING OF THE TOBACCO HORNWORM, <u>MANDUCA</u> <u>SEXTA</u>

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AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

    The development of cuticular mechanoreceptors (campaniform sensilla)

on the wing of Manduca sexta was investigated using pupae of known ages.

Sensilla along the wing were surveyed electrophysiologically for responsive-

ness to mechanical stimulation and were examined with scanning and trans-

mission electron microscopy. Sensilla near the middle of the wing's proximal-

distal axis became responsive the day before eclosion, whereas sensilla

along the distal margin did not become responsive until late on the day of

eclosion. Scanning electron microscopy showed that the cuticular dome

and rim of the distal sensilla are fully formed by Day 16 of pharate adult

development. Transmission electron microscopy indicated that the sensory

process appears morphologically complete approximately 4 days before eclosion,

or 3 days before the receptors become responsive to stimulation. The only

morphological difference observed between nonfunctional and functional receptors

was a receptor lymph space in functional sensilla. Results suggest the

existence of a proximal-distal developmental gradient along the wing and

support the hypothesis that the receptor lymph space is actively involved

in the process of sensory transduction.